Relationship between magnitude and direction of asymmetries in facial and limb traits in horses and ponies.

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Abstract

Directionality of limb and facial asymmetries in non-elite equine populations has been previously reported with results indicating strong similarities to those reported in racehorses. This investigation aimed to consider the relationship between the magnitude of the asymmetries presented within the general equine population, and their previously reported directionality. Direct measurements of 15 bilateral traits (four facial and 11 limb) were captured from a mixed population of 100 horses and ponies. The pooled (whole) population was considered further as horse (withers height >148cm) and pony (withers height ≤148cm) groupings. Each of the three groups were further sub-divided for each trait, into individuals presenting with larger left or larger right sides. Asymmetries were compared as mean asymmetries and as percentages of the trait size at each grouping level. Asymmetry magnitudes were largely reflective of the directional asymmetries previously recorded. Both the horse and pony groups presented with significantly longer right side third metacarpal ($P \leq 0.001$) and third metatarsal ($P \leq 0.05$ and $P \leq 0.001$) bones, whilst in the horse group, the left fore proximal phalanx was both longer and wider compared to the right ($P \leq 0.001$ and $P \leq 0.05$). This pattern is reflective of the biomechanical preference for left lead anticlockwise) canter, previously only observed in racehorses. The proximal phalanx of the forelimb potentially compensates for the higher loading forces associated with the lead forelimb. When scaled as percentages of trait size, the asymmetry magnitudes largely reflected those reported in humans, suggesting similar criteria could be applied when considering stock selection and controlling for injury predisposition in horses.

Key words: Equine, Asymmetry, Conformation, Laterality, Distal limb

1.0 Introduction

The symmetry of an individual is suggested to be a reflection of their ability to resist environmental or genetic stressors during growth and development [1–3]. As symmetry is portrayed physically through phenotypic expression, the degree of lateral asymmetry within functional and non-functional traits is commonly used to obtain measures of asymmetry [4]. Directional and fluctuating asymmetry are the two most important measures of asymmetry [5]. Directional asymmetry (DA), either at an individual or population level, is reflective of the mean of a specific trait being significantly larger on one side compared to the other, creating an anatomical directional bias [4] with a potential influence on physiological laterality. A positive directional asymmetry value depicts the left side being larger whereas a negative directional asymmetry value indicates a larger right side [6]. Fluctuating asymmetry (FA) is a population level measure of the random deviations across individuals resulting from bilateral trait symmetry, indicated by a lack of directional bias between the left and right sides. FA is considered a measure of developmental homeostasis at both an individual and population level [5,7,8]. The presence of inherent symmetry across a species indicates the presence of a superior genotype, potentially enhanced brain development, higher levels of physiological health, and has been linked with superior cognitive capacity; representing an evolutionary advantage for the individual [9–11]. In contrast, a high degree of functional asymmetry can be detrimental and is thought to increase the potential for poor performance and injury acquisition in humans through unequal loading and movement instability [11,12].

Asymmetry in the adult *Equid* has the potential to be related to inherent cognitive, motor or developmental lateralisation, or it can be acquired as a result of injury or training demands and management practices [13–15]. Symmetry has been linked with superior performance in Thoroughbred racehorses [16] and event horses [17]. For example, poor performing racehorses demonstrate greater asymmetry in *Tuber sacrale* height [18], whilst lower ranking Thoroughbred racehorses exhibit greater asymmetry of non-functional facial traits and functional limb traits than their high performing counterparts [16]. Limited research has examined DA outside of Thoroughbred racehorse populations. Leśniak [15], established the presence of DA's within the same population of non-racing horses and ponies used within this current study and reported that those DA's were exhibited in similar bilateral traits to those previously reported in Thoroughbred racehorses [14,16]. However, whilst DA's were identified, to be able to establish the potential impact of these asymmetries on the functional capacity of individuals, the magnitude of the DA should also be established. This study aimed to compare asymmetry magnitudes of functional and non-functional traits in a non-racing population of horses. We hypothesised that, for each trait, the side with the greater frequency of DA, as determined by Leśniak [15], would present with the lower asymmetry magnitudes.

2.0 Methods

2.1 Study population

The study population consisted of 100 horses and ponies of mixed age, breed and sex from two equestrian establishments in Gloucestershire, UK. None of the population had an elite[1] competitive record and all were over five years of age to eliminate age related developmental symmetry fluctuations [19]. The population was selected via convenience sampling and analysed as three groups: pooled data (*n=100*), horses (withers height >148cm[2]; *n*=57) and ponies (withers height ≤148cm; *n*=43).

2.2 Measurement method

Direct measurements of 11 functional and four non-functional bilateral traits (Table 1) were determined using Invicta metric calipers (1 mm accuracy) (Invicta Education, Bicester, UK). The measurement methodology is described in detail within the previous directional asymmetry analysis of these data [15]. Two observers repeated the measurements for selected horses following the same protocol and inter-observer repeatability (r) was calculated using the following equation [20]:

$$r = s^2A / (s^2 + s^2A)$$

Where s^2A is the between-group variance and s^2 is the within-group variance

[1] elite was defined as not having competed within any discipline above a National Governing Body affiliated novice level

[2] The Fédération Équestre Internationale define a pony as being 'up to 148cm at the withers'

2.3 Data analysis

Frequency values of directional asymmetries (DA) previously determined by Leśniak [15] using the following equation, were utilised within analysis of the three groups.

$$DA = L - R$$

Where L= Left side measurement, R= right side measurement. A positive directional asymmetry indicated a left side bias, and a negative directional asymmetry indicated a right side bias. Absolute asymmetry (AA) (mm), considered within the current analysis, refers to the degree of asymmetry irrespective of the direction. To take into consideration the varying heights of the horses within the study population, relative asymmetries for each trait were also considered as a percentage of the mean trait size (TA%) using the equation:

$$TA = \left\{\frac{(L - R)}{((L + R) * 0.5)}\right\} * 100$$

Data within each group were analysed following removal of outliers (±2 s.d.) and determination of distribution using the Kolmogorov-Smirnov test. Mean left and right side measurements were compared within each group for each of the traits. Reliability of the measurement protocol was undertaken through the use of a repeated measures one-way ANOVA and 2 way mixed methods inter-rater reliability analysis to determine if significant variance occurred between the three repeated measurements.

2.3.1 Pooled, pony and horse group analysis

Following distribution analysis, Friedman's tests with *post-hoc* Wilcoxon signed rank analyses determined whether, within each of the three groups, specific traits presented with significantly larger TA% or AA. Differences in TA% and AA between the three groups were established through a series of Kruskal-Wallis tests with Mann-Whitney U *post-hoc* analyses for each trait.

2.3.2 Left-larger, right-larger, sub-group analysis

Further analysis was undertaken to determine whether those traits with a significant directional bias to be larger on one side [15] also demonstrated significantly larger/ smaller asymmetries compared to the other side. Within each group (pooled, horse, pony), bilateral pairings for each trait, were clustered according to whether they were larger on the left (LL) or on the right (RL) (DA as previously determined by Leśniak [15]). LL and RL sub-groups were therefore, established for the pooled, pony and horse groupings (Figure 1).

To determine whether AA and TA% differed significantly between the LL and RL sub-groups of each trait, either the paired t-test or the Wilcoxon's Signed rank tests were employed, dependent on normality distribution within each trait. To determine whether AA and TA% of the LL and RL sub-groups differed significantly between population groups (pooled, horse and pony), either the Independent samples t-test or Mann-Whitney U test were used dependent on normality distribution within each trait. Thus, data analysis enabled asymmetry magnitudes to be determined both with and without a directional context. All analyses were performed using the statistical analysis software SPSS (IBM SPSS version 24) with the significance level set at $P<0.05$ throughout.

3.0 Results

3.1 Reliability of measurement results

No differences were reported between the three measurements of the traits investigated with the exception of HPP length (HPP; Anova: $P\leq0.05$); inter-rate repeatability was excellent for all traits measured (ICC: 0.99–1.00), and therefore data are reported with confidence. Directional asymmetry frequencies were reflective of those reported in Leśniak [15].

3.2 Group level analysis; pooled, pony and horse.

Statistically significant differences between left and right measurement means were determined for six traits within the pooled group (Table 2). The largest asymmetries as a percentage of trait size (TA%) were expressed by FPP length, HPP length and nostril width (Table 3); each demonstrating significant differences from the TA% of at least seven other bilateral traits (Table 4). Analysis of means revealed significant differences between left and right within the pony group.

Mean measurements of the MC3 and MT3 lengths, and carpal and tarsal widths were significantly larger on the right ($P\leq0.001$); whereas MT3 width ($P\leq0.05$) and pinna width ($P\leq0.001$) were significantly larger on the left.

The largest TA% values within the pony group were expressed by the FPP length, HPP length and nostril width (Table 3); similar to the result of the pooled group. Only TA% of nostril width stood out as being very highly significantly different (*P*≤0.001) from the majority of the other bilateral traits (Table 4). Pinna length TA% was significantly lower (*P*≤0.05) than nine of the other 14 bilateral traits (Table 4)

Statistically significant differences between left vs right means were established for four traits within the horse group; the left limb demonstrated larger FPP length (*P*≤0.001) and width (*P*≤0.05) whilst the right limb presented with larger MC3 (*P*≤0.001) and MT3 length (*P*≤0.05). No significant differences were recorded for facial traits.

TA% was greatest for nostril width ($\bar{x}$=6.99% compared to between 1.15-4.21% within the other traits). Akin to the pony group, this was the only trait whose TA% was significantly different (*P*≤0.001) from the majority of the other bilateral traits (Table 4). TA% for pinna length, pinna width, MT3 and HPP length, and tarsal width were relatively low; however, they differed significantly (*P*≤0.05) from the majority of the other bilateral traits (Table 4).

Cross-group analysis of TA% and AA did not identify differences between the three groups for facial traits; significant differences for the four limb traits were, however, determined. AA of MC3 width was significantly lower for ponies than for either the horse (*P*≤0.001) or pooled (*P*≤0.05) groups. TA% differed between all three groups for MT3 length; differences between the horse (*P*≤0.001) and pony (*P*≤0.001) groups were larger than their individual differences with the pooled group (*P*≤0.05) (Table 3).

MC3 length TA% was significantly lower in horses than in ponies (*P*≤0.05); as was FPP length (*P*≤0.001). The latter was likely responsible for the pooled group exhibiting a significantly (*P*≤0.05) greater TA% for this trait than the horse group.

3.3 LL and RL sub-group differences

Analysis of the LL and RL sub-groups, established that pinna width AA was significantly larger within the LL sub-group (P≤0.05) within the pooled group; no significant differences in TA% between sub-groups were reported. AA of HPP length and tarsal width were significantly larger (P≤0.05) in the LL sub-group. However, AA of MC3 length and carpal width were significantly larger (P≤0.05) in the RL sub-group. TA% was greater within the RL sub-group for FPP length (P≤0.05), MC3 length (P≤0.05), MT3 length (P≤0.001) and carpal width (P≤0.01).

Within the pony group, AA ($P \leq 0.01$) and TA% ($P \leq 0.05$) of pinna width and AA of nostril width ($P \leq 0.05$) were significantly larger for the LL sub-group. AA for MC3 length ($P \leq 0.01$) and MT3 length ($P \leq 0.01$) was significantly greater in the RL sub-group. A similar pattern in TA% was reported for both these traits; MC3 length ($P \leq 0.001$), MT3 length ($P \leq 0.05$).

Unlike the pooled and the pony groups, no significant differences in AA or TA% of the facial traits were identified in the either of the horse sub-groupings. AA was larger in the RL sub-group for MC3 length ($P \leq 0.05$), MT3 length ($P \leq 0.01$) and carpal width ($P \leq 0.01$). However, AA of the FPP width ($P \leq 0.05$) and tarsal width ($P \leq 0.01$) was greater in the LL sub-group. TA% was greater for the RL sub-group for MC3 length ($P \leq 0.05$), MT3 length ($P \leq 0.05$) and carpal width ($P \leq 0.01$). However, TA% in the LL sub-group were greater for FPP width ($P \leq 0.05$), HPP length ($P \leq 0.05$) and tarsal width ($P \leq 0.01$).

4.0 Discussion

This study aimed to determine whether directionality of an asymmetry was associated with its magnitude. For structures such as the appendages and facial features, perfect symmetry is traditionally considered the optimum status for an individual's fitness [21]. Therefore within a symmetrically orientated population, a mean of zero and a normal distribution around this mean should be observed for left-right differences within bilateral traits [22]. In reality, directional asymmetry has been widely reported across species and for various specific traits [4,5,16]. The directionality reported in the initial analysis of the current data [15] suggests an inherent asymmetry within *Equidae* for specific bilateral traits, but one that is not similarly presented across both horses and ponies for all traits. Group size differences between the previously reported directionality and the current study are reflective of the removal of outliers within the current analysis; a procedure not necessary for the analysis of directional frequencies. The degree, or magnitude of the asymmetries presented varied dependent on; grouping, sub-grouping and the specific trait measured; this was particularly so for TA%. Whether a greater DA bias also reflects a greater magnitude of asymmetry, depends on the individual trait; resulting in rejection of the initial hypothesis as no single overarching model was identified.

4.1 Asymmetry range identification

From the results of the study, the authors further attempted to propose 'normal' asymmetry ranges for the functional and non-functional traits evaluated. Significant differences between horse and pony groupings for a number of the bilateral traits investigated, indicated that no single 'normal' asymmetry range could be determined. Fluctuating asymmetry (FA) exhibited by a population is suggested to be in the region of 1-2% of trait size [23]; this value is more reflective of functional traits. Non-functional traits, such as the upper body traits in humans, more commonly exhibit variability in the region of 3-4% [24]. When split in to LL and RL sub-groups, the TA% in horses and ponies was much higher than the previously reported values. However, when considered within the horse, pony and pooled groupings (Table 4) results are much more aligned to values considered within the normal range in human studies. These findings reinforce the need to consider horses and ponies separately within research and practice.

Interestingly, and despite width measurements of the traits being highly repeatable, it was the only proximal phalangeal traits that exceed the 1-2% range within the functional traits investigated. Osteogenic changes to width dimensions may reflect a compensatory mechanism in response to asymmetries of other directly, or indirectly linked, regions; such as length dimensions. However, the unexpectedly high TA% for PP lengths may also be influenced by measurement difficulties within this location. We propose that the 'normal' asymmetry range applied within human asymmetry research could also be applied, across species, to *Equidae*. Therefore, values significantly above the 1-2% for functional traits might both reflect compensatory mechanisms for other existing asymmetries and imply a functional disadvantage or increased risk of injury. It would be pertinent, when selecting stock as performance horses, to select those with the lowest degree of TA% as these individuals are more likely to have a greater career longevity due to a reduced risk of compensatory injury.

4.2 Facial (non-functional traits)

Measurement of facial traits allows inclusion of bilateral traits not functional in locomotion. Previous research suggests asymmetries of non-functional traits may be more common, and of greater magnitude, than those found within functional bilateral traits, where symmetry would be a biomechanical advantage [19,25]. Asymmetry as a percentage of traits size (TA%) for the widths of both the nostrils and the pinna, reflect this theory within the current investigation. Assimilation of the current findings with those of Leśniak [15] evidences a left directional pinna bias, particularly in ponies (Figures 2 to 4). Although pinna length asymmetries were frequent, they were generally only small in magnitude; particularly when considered as TA%. Left pinna length measurements were larger, albeit the difference was non-significant. Furthermore, asymmetries were greater for those exhibiting left-side bias for this bilateral trait; contradicting our hypothesis. Despite little functional significance, pinna asymmetries in humans are speculated to reflect conditions such as auditory canal defects and conductive deafness [26] and may consequentially influence balance and proprioception abilities.

As previously proposed in Leśniak [15], a 'normal' pinna asymmetry for *Equidae* is potentially a bias for the left side to be longer; similar to that observed in human females [26]. A slight left DA bias of pinna length in primate mothers has been linked to the production of more symmetric offspring [26] in terms of pinna length; greater symmetry being considered the phenotype optimum. Pinna asymmetry has also been implicated in the morphological expression of the developmental stability of the cerebral hemispheres [26]; a region involved in emotional decoding of audio and visual signals from left-side receptors. Disruption to optimal cerebral hemispheric development has the potential to negatively affect auditory perception by impacting the passage of sensory information inevitably influencing sensory decoding of audio [26,27] and indeed non-verbal stimuli within this region [28].

Despite the overt left directional frequency bias determined in Leśniak [15] for nostril traits, the difference between the left and right measurements of the traits within the current analysis, was not significant. TA% was however, significantly larger for nostril width than for all other traits within all groupings; particularly within the pony group. Although every effort was made not to evoke a response, flaring of the nostrils due to the novelty of the calipers could have resulted in the high TA% levels. The nostril width measurements were taken from the region of the nostril least likely to be affected by flaring but, due to the naturally inquisitive and cautious nature of *Equidae*, possible errors due to nostril flare cannot be discounted. Furthermore, nostril asymmetry within the pony group could, as with pinna asymmetry, be linked to brain lateralisation. McGreevy and Rogers [29] reported a right nostril preference during an olfactory stimulus test using stallion faeces within a mixed sex population of horses (n=106). Their findings suggest nostril use can be an indicator of sensory lateralisation to novel stimuli and therefore, as olfactory stimuli are processed ipsilaterally, a right cerebral dominance. Higher asymmetry magnitude in LL ponies could be a reflection of a greater sensory awareness due their more native bloodlines. Conversely, the greater comparative symmetry of nostril width in horses may reflect the more notable selective breeding for athletic capability. Aerobic capacity being a key contributor to athletic performance and one which can be negatively impacted by impedance of ventilation [30].

4.3 Limb (functional) traits

An investigation into the prevalence and characteristics of asymmetries in humans by Trivers et al. [19] suggested that even small limb asymmetries could result in adverse biomechanical consequences. As quadrupeds, horses are better able to compensate for limb length discrepancies than bipeds. However, such alterations in biomechanics would still result in a change to the kinematics and kinetics of the stride, potentially influencing career longevity. The level of the asymmetries in the functional traits within the current study is within the range of millimeters. As such this may be of little consequence in normal loading, such as stance where the horse rarely stands with both contralateral limbs at the same height due to uneven bedding, pasture, etc. However, it may become of greater significance during repeated intensive loading, such as when being worked, or it may be reflective of further underlying functional vulnerabilities.

Whilst FPP traits recorded a left directionality bias in terms of frequency (albeit only significant in the horse group), magnitude bias was overtly different between the horse and pony groups. Within the horse group, both AA and TA% were greater in the LL sub-group. However, all variables were greater on the right in the pony group, except for AA of the FPP length. This further reflected differences between these two groupings. The current study supported the previously determined [15] right-side directional bias of MC3 length in both horses and ponies by exposing larger right-side mean measurements for all groups. Ponies demonstrated greater TA% for MC3 length; however, at a sub-group level, AA and TA% were also greater within the RL sub-group for horses. This reflects that, in addition to an individual bias towards a longer right side, the asymmetry magnitude for those with a right-side bias was greater than horses presenting a left-side bias; again contradicting our initial hypothesis.

A directionality bias for MC3 width was not previously identified in Leśniak [15] and no significant differences were found within the intragroup analysis of the current study. The significantly lower AA of MC3 width of the pony group, in comparison to the pooled and horse groups was however surprising as the opposite had been predicted. To date, despite their high level of repeatability, MC3 and MT3 width dimensions have not been investigated within other equine studies. Asymmetry magnitudes of MC3 length demonstrated a similar right side bias to the previously determined DA. Adaptation to MC3 length asymmetries are likely to manifest in either the width dimension of bones other than MC3, metacarpophalangeal joint angulations, or hoof conformation. Wilson et al. [31] reported such hoof conformation compensations noting an increased hoof spread (difference between the coronet band and hoof base circumferences) within the shorter limb, proposing this as a consequence of the increased loading it is subjected to.

For a number of traits where differences in horse and pony groupings were observed, (e.g. carpal width, HPP length and MT3 width), the asymmetry magnitudes expressed by the horse group was sufficient to influence the pooled data group. These discrepancies suggest that the amalgamation of data as a pooled group does not facilitate recognition of the stand-alone results observed within the sub-groups.

Overall the DA and asymmetry magnitude data for both the horse and pony groups indicates that the MC3 and MT3 lengths of the right leg are longer, whilst the FPP presents greater width and length in the left limb. Although not statistically significant, the joint dimensions of the carpal joint presented with a bias for a larger right side, whilst the tarsal joints were larger on the left. The ipsilateral long bone asymmetry, combined with a potential predisposition for left lateralisation in the canter [32] may account for the bias for larger left FPP traits. In an asymmetric gait, such as the canter and gallop, the leading forelimb sustains higher ground reaction forces on landing than the leading hindlimb [33]; the larger geometry of the FPP may offer a compensatory mechanism for these increased loads.

The study hypothesis proposed that the side with the greater frequency of DA in terms of MT3 length and width would present with the lower asymmetry magnitudes.

Asymmetry magnitude, both as AA and as TA%, was significantly larger for the RL compared to the LL sub-groups. Therefore, although the two sub-groups were similar in size, those with a longer right MT3 presented with a much greater asymmetry. This could be reflective of the lack of quality breeding and the influence of pony cross-breeding within the population of the current study thereby increasing the level of heterozygosity. Despite the lack of expected bilateral symmetry observed, the longer right MC3 does reflect the inherent bias for Equidae to canter with a leading left forelimb [32] and a potential biomechanical advantage for running clockwise. The greater FPP length and width dimensions may have manifested as compensatory mechanisms to accommodate the increased loading and provide additional stability to the more heavily loaded left limb.

Differences in asymmetry magnitudes between horses and ponies, and their respective sub-groups, may be a consequence of the difference in breeding strategies between the two groups. Breeding of horses is managed more intensively than that of ponies, with many horses having Thoroughbred influences within their bloodlines. Various modern horse breeds, such as the Irish Sports Horse and the European Warmbloods, with lighter frames than their ancestral counterparts, have deep-seated Thoroughbred lineages [34,35]. A degree of inbreeding is known to exist within the Thoroughbred industry as a result of breeding strategies employed [36]. Reduced heterozygosity has, in other species, been linked to developmental instability and therefore an increase in asymmetry within bilateral traits [37–40]. Reduced heterozygosity may go some way to explain the differences reported between horses and ponies in the current findings, particularly with regard to the facial traits. However, phenotypic consequences of breeding strategies, as opposed to heterozygosity, may better explain the propensity for horses to present with greater symmetry of limb bone length, whilst the width of the same bones is more symmetrical in ponies.

5.0 Conclusion

Where directional bias exists within trait dimensions, the asymmetry magnitude will be larger within the side exhibiting the greater bias. The range reported within this *Equidae* population was reflective of those reported in human studies; however, the biomechanical significance and potential for injury predisposition remains undetermined as yet. Whilst there were some similarities within horses and ponies, the significant differences between the two, reinforces the existence of more diverse phenotypical differences than height alone. Whether this disparity is exacerbated through the difference in breeding strategies, or is inherent to the informal 'horse' and 'pony' divisions in the *Equus caballus* taxonomy, remains to be investigated.

Author contributions:

KL designed the study, planned the investigations, collected, and analysed the data. KL and JW interpreted the results. KL wrote the manuscript and JW critically reviewed the manuscript.

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Table 1. Bilateral traits measured including description and abbreviations

Abbrev.	Trait	Description
MC3L MT3L	Third metacarpal length & third metatarsal length	Measured laterally from the 'V' formed by the overlap of the annular ligament over the superficial digital flexor tendon at the distal portion of the limb, to the protrusion of the fourth metacarpal/ metatarsal at the proximal region of the distal limb
MC3W MT3W	Third metacarpal width & third metatarsal width	Measured on the horizontal axis half way between the carpometacarpal / tarsometatarsal joint and the metacarpophalangeal /metatarsophalangeal joints
FPPL HPPL	Fore proximal phalanx length & hind proximal phalanx length	Measured laterally from the protuberance of the lateral cartilage of the distal phalanx to the lateral protrusion made by the proximal condyle of the proximal phalanx
FPPW HPPW	Fore proximal phalanx width & hind proximal phalanx width	Measured horizontally at the narrowest point of the phalanx
CW	Carpal joint width	Measured horizontally from the medial to the lateral aspects of the inter-carpal joint
CD	Carpal joint depth	Measured laterally from the dorsal aspect of the intermediate carpal bone to the palmer aspect of the accessory carpal bone
TW	Tarsal joint width	Measured horizontally from the medial to the lateral aspects of the tarsocrural joint
PL	Pinna length	Measured from the point at the summit of the pinna to the inverted point at the base of the pinna
PW	Pinna width	Measured from the medial to the lateral aspect of the pinna at the midpoint of its length.
NL	Nostril length	Measured from the top of the fold on the medial aspect of the nostril to the lowest point of the nostril
NW	Nostril widths	The width of the nostrils was measured horizontally from the alar fold on the medial aspect to reduce the impact of nasal flaring, to the lateral border of the nostril

Table 2. Mean left-right measurements for the pooled group (mm)

Bilateral trait	$\bar{x}$ left side	$\bar{x}$ right side	Significance level
Pinna length	136.35	136.03	$P\geq0.05$
Pinna width	54.67	53.99	$P\leq0.001$
Nostril length	55.22	55.05	$P\geq0.05$
Nostril width	21.30	20.85	$P\geq0.05$
Fore proximal phalanx length	56.46	55.75	$P\leq0.05$
Fore proximal phalanx width	51.84	51.36	$P\leq0.05$
Third metacarpal length	171.25	172.84	$P\leq0.001$
Third metacarpal width	43.01	43.00	$P\geq0.05$
Carpal width	94.65	95.48	$P\leq0.001$
Carpal depth	102.82	103.08	$P\geq0.05$
Hind proximal phalanx length	56.78	56.39	$P\geq0.05$
Hind proximal phalanx width	53.66	53.42	$P\geq0.05$
Third metatarsal length	209.04	210.78	$P\leq0.001$
Third metatarsal width	41.61	41.44	$P\geq0.05$
Tarsal width	93.84	94.03	$P\geq0.05$

Table 3. Total asymmetry (AA) and as a percentage of trait size (TA%) for the three groups and their subgroups

BILATERAL TRAIT		POOLED		PONY		HORSE		POOLED				PONY				HORSE			
								AA		TA%		AA		TA%		AA		TA%	
		AA	TA%	AA	TA%	AA	TA%	LL	RL	LL	RL	LL	RL	LL	RL	LL	RL	LL	RL
Pinna	Length	1.67	1.23	1.36	1.13	1.83	1.19	2.08	1.67	1.52	1.23	1.81	1.32	1.54	1.04	2.57	1.73	1.61	1.17
	Width	1.67	3.06	1.45	2.97	1.87	3.20	2.08	1.52	3.76	2.90	1.85	0.87	3.71	1.93	2.32	2.33	3.80	3.41
Nostril	Length	1.58	2.92	1.40	2.92	1.52	2.57	1.72	1.79	3.13	3.39	1.48	1.54	3.23	3.07	1.62	1.80	2.65	3.16
	Width	1.79	9.15	2.07	11.95	1.53	6.99	2.30	1.70	11.15	9.39	3.06	1.77	14.39	12.04	1.98	1.65	8.84	7.02
Fore proximal phalanx	Length	2.42	4.82	2.67	6.57	1.94	2.96	2.44	2.82	4.57	6.16	2.75	2.72	6.47	7.17	2.38	1.83	3.68	2.65
	Width	1.59	3.06	1.15	2.56	1.83	3.26	2.02	1.65	3.81	3.31	1.28	1.57	2.80	3.59	2.51	1.60	4.49	2.81
Third metacarpal	Length	2.78	1.74	2.65	2.00	2.63	1.37	2.35	3.30	1.37	2.09	1.43	3.28	1.02	2.49	1.58	3.25	0.82	1.69
	Width	0.72	1.66	0.44	1.11	0.83	1.80	0.95	1.02	2.17	2.37	0.75	0.72	1.90	1.85	1.03	1.02	2.18	2.10
Carpal	Width	1.70	1.85	1.76	2.20	1.43	1.37	1.51	2.34	1.56	2.59	1.68	2.38	2.14	2.97	1.20	2.30	1.12	2.22
	Depth	1.64	1.62	1.45	1.59	2.08	1.89	1.80	1.88	1.77	1.86	1.49	1.90	1.69	2.04	2.00	2.41	1.81	2.19
Hind proximal phalanx	Length	2.72	5.17	2.64	6.23	2.69	4.21	3.38	2.33	6.21	4.60	3.51	2.28	7.69	5.59	3.31	2.16	5.15	3.41
	Width	1.60	3.03	1.43	3.04	1.74	2.90	1.82	1.92	3.41	3.69	1.61	1.80	3.41	3.85	2.20	1.78	3.67	2.94
Third metatarsal	Length	3.22	1.67	3.82	2.31	2.71	1.15	2.09	2.90	1.09	2.03	1.83	4.70	1.46	2.69	2.00	3.34	0.82	1.44
	Width	0.71	1.74	0.62	1.72	0.82	1.78	0.90	1.03	2.20	2.52	0.96	0.79	2.66	2.26	1.03	1.00	2.26	2.14
Tarsal width	Width	1.36	1.49	1.07	1.31	1.44	1.40	1.91	1.37	1.94	1.58	0.80	1.31	0.93	1.16	2.16	1.22	2.07	1.20

Table 3 depicts the absolute asymmetry (AA) (mm) and asymmetry as a percentage of trait size (TA%) of each of the four non-functional (facial) and eleven functional bilateral traits. Results are presented for the three main groups (pooled, pony and horse), as well as for each of their left-larger (LL) and right-larger (RL) subgroupings.

Table 4. Differences between traits as a percentage of trait size for the pooled, pony and horse groupings. A full key of bilateral trait abbreviations can be found in Table 1

	PL	PW	NL	NW	MC3L	MC3W	MT3L	MT3W	FPPL	FPPW	HPPL	HPPW	CD	CW	TW
PL		A** H**	A** P H**	A** P** H**	P**	H**	P**	P* H*	A** P** H**	A** H**	A** P** H**	A P* H**		P**	
PW				A P**	H**	A H	A* H**	A H					A	A H**	A* H**
NL				A** P** H**			H**								H**
NW					A** H**	A** P** H**	A** H**	A** P** H**		A** P** H**		A** P** H**	A** P**	A** H**	A** P** H**
MC3L									A**		A** H**				
MC3W								H	A**		A** H**				H
MT3L									A** H**	H**	A** H**	H**	P		P*
MT3W									A**		A** H**				
FPPL												A	A** P	A**	A** P* H**
FPPW														H*	H**
HPPL												A	A** P	A** H**	A** P* H**
HPPW															H**
CD															
CW															
TW															

A	Pooled
H	Horses
P	Ponies

* P≤0.01, ** *P*≤0.001,

PL = Pinna length; PW = pinna width; NL = Nostril length; NW = Nostril width; MC3L = Third metacarpal length; MC3W = Third metacarpal width; MT3L = Third metatarsal length; MT3W = Third metatarsal width; FPPL = Fore proximal phalanx length; FPPW = Fore proximal phalanx width; HPPL = Hind proximal phalanx length; HPPW = Hind proximal phalanx width; CD = Carpal depth; CW = Carpal width; TW = Tarsal width.

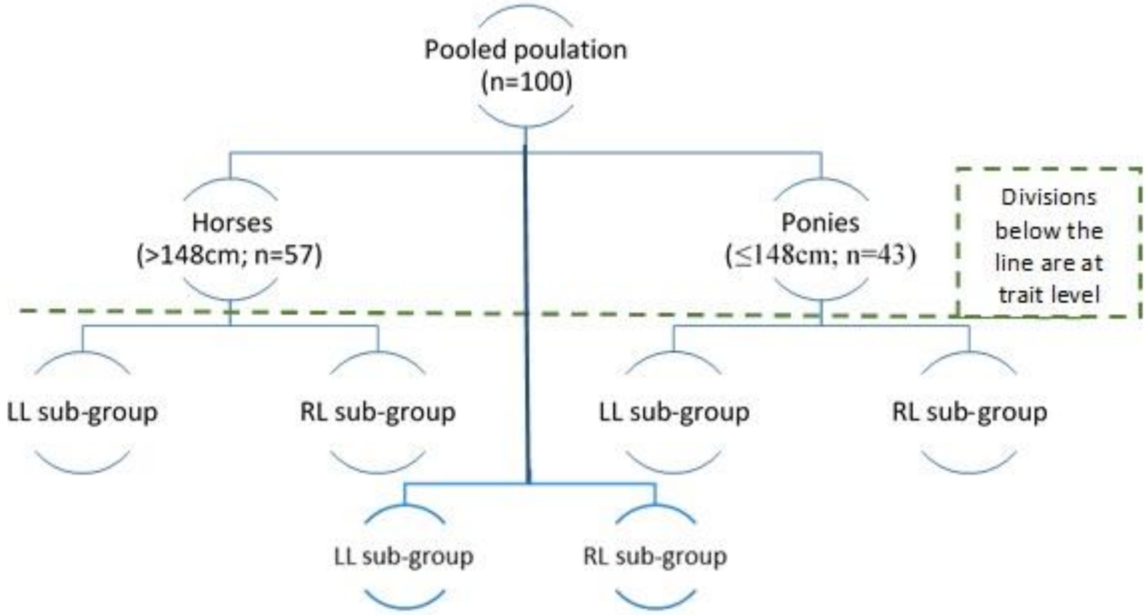

Figure 1: Divisions of the sample population in to groups (pooled, horse and pony) and into LL and RL sub-groups at trait level.

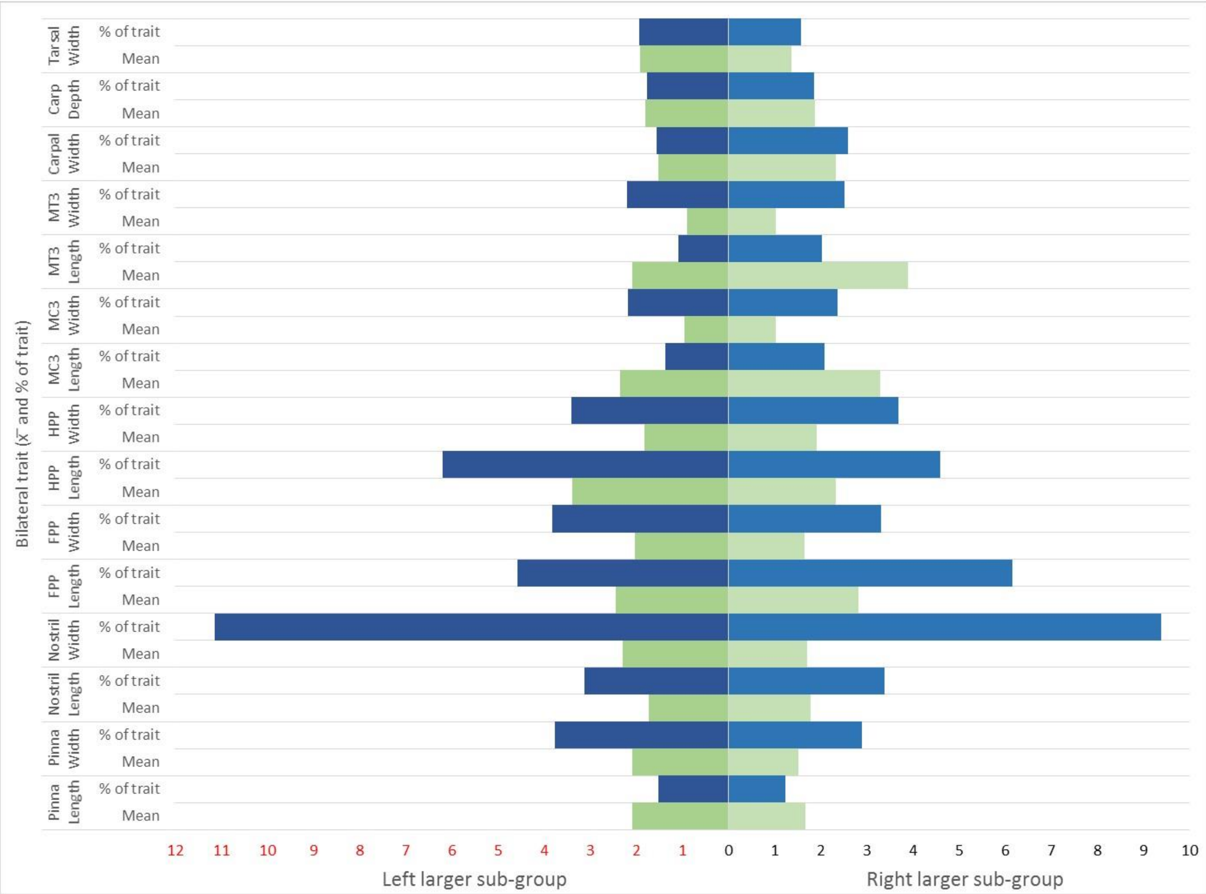

Figure 2: Pooled group LL and RL subgroupings illustrating the distribution of AA and TA% with respect to the directionality determined in Leśniak (2013). (DA* - Scale to be multiplied by 10 to give frequency as a percentage i.e. 5 = DA 50%; mean to be multiplied by 100 to equate to SI units (m))

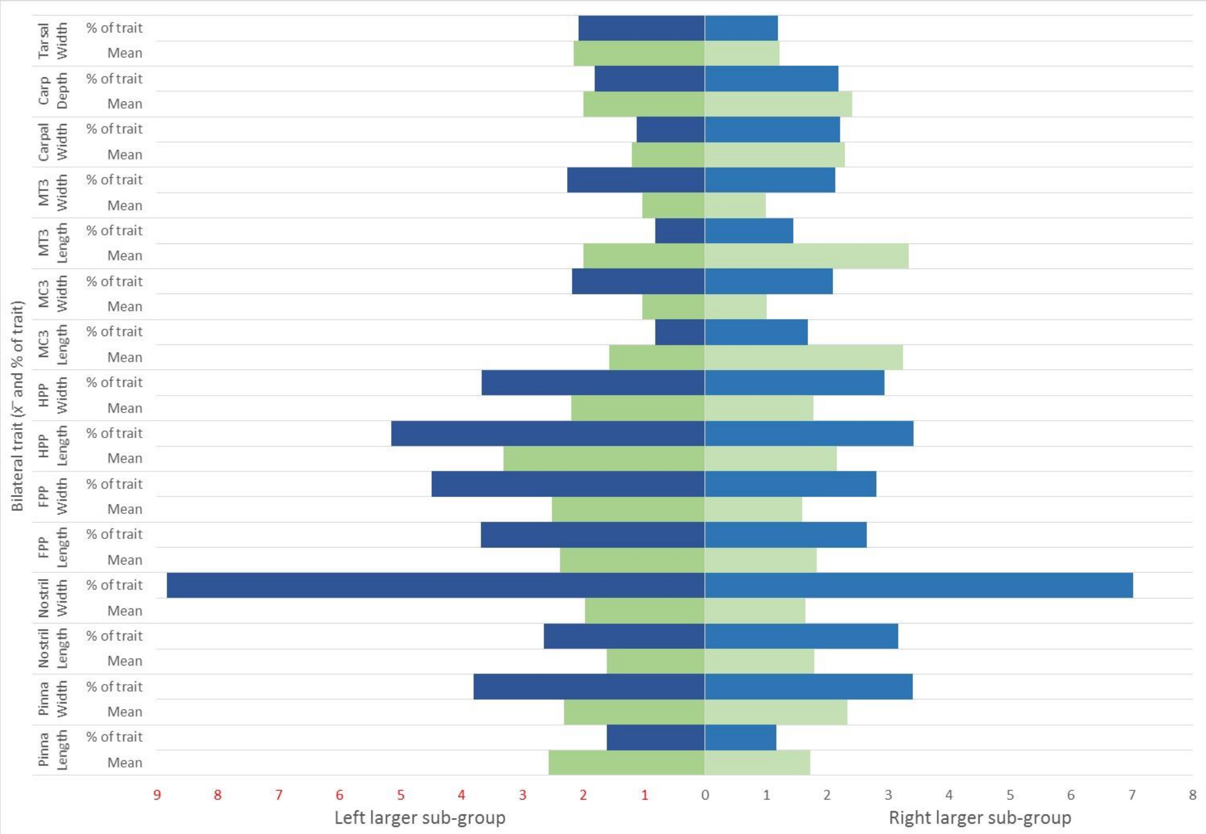

Figure 3: Horse group LL and RL subgroupings illustrating the distribution of AA and TA% with respect to the directionality determined in Leśniak (2013). (DA* - Scale to be multiplied by 10 to give frequency as a percentage i.e. 5 = DA 50%; mean to be multiplied by 100 to equate to SI units (m)).

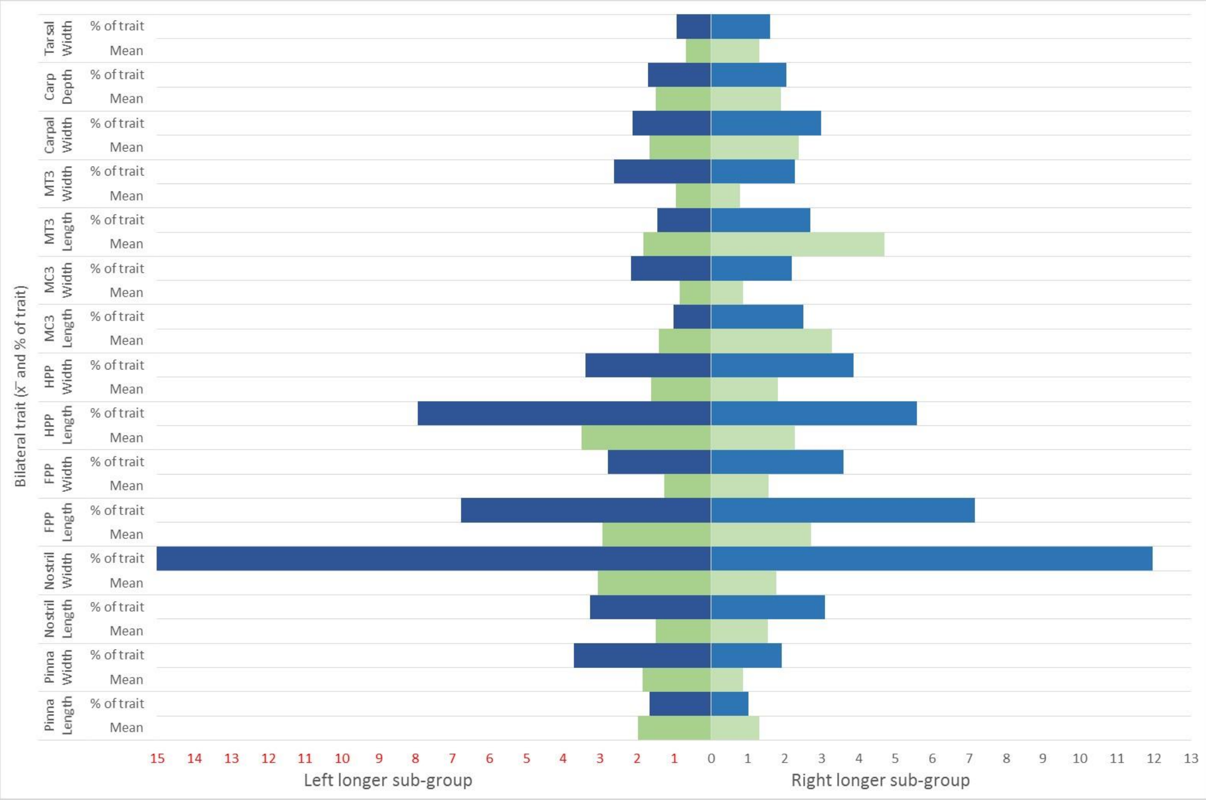

Figure 4: Pony group LL and RL subgroupings illustrating the distribution of AA and TA% with respect to the directionality determined in Leśniak (2013). (DA* - Scale to be multiplied by 10 to give frequency as a percentage i.e. 5 = DA 50%; mean to be multiplied by 100 to equate to SI units (m)).